Rapid *In Vitro* Propagation of *Hornstedtia reticulata* (K. Schum.) K. Schum.

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Abstract

Seeds of *Hornstedtia reticulata* (K. Schum.) K. Schum. collected from the wild were double surface sterilised with 30% Clorox, followed by 15% Clorox, each for 20 minutes. The sterilised seeds were sown on Gamborg B5 medium. The meristems of 12 weeks old seedlings, including the basal parts of leaf sheath, were used to induce multiple shoots in Gamborg B5 media incorporated with 6-benzylaminopurine (BAP) alone (2mg/L and 3mg/L) and in combination with α-naphthalena acetic acid (NAA) at different concentrations (0.5mg/L and 0.1mg/L). Observation showed that all the treatments were able to produce multiple shoots while the highest number of shoots was obtained from explants that were treated with 3mg/L BAP after three subcultures.

Introduction

Hornstedtia Retz. is a well-defined genus characterized by the rigid involucre of sterile bracts, which encloses the entire inflorescence from the uppermost part of the open flowers. Valeton (Bull. Jard. Bot Buitenz. Ser. 3, 3: 150-179, 1921) cited by Smith (1985) had subdivided *Hornstedtia* into three subgenera, *Hornstedtia*, *Elettariostemon* and *Rosianthus*. The Bornean plants all fall within the first two groups.

Hornstedtia reticulata (K. Schum.) K. Schum. is very distinctive. The cyathiform inflorescence is borne on stilt roots, and the sterile bracts, which are the most strongly reticulated of all Bornean *Hornstedtia*, are scabrid to the touch. Study on species of *Hornstedtia* is scarce; hence, information beyond taxonomy study is still unavailable.

Up to this moment, several species of Zingiberaceae with established

uses as condiments, spices and as ornamental plants have been investigated for *in vitro* multiplication (Borthakur *et al.*, 1999; Jasrai *et al.*, 2000; Salvi *et al.*, 2000; Rout *et al.*, 2001; Khatun *et al.*, 2003; Prakash *et al.*, 2004; Wondyifraw *et al.*, 2004). So far, no successful micropropagation protocol for *Hornstedtia* spp. has been reported. For this reason, this study served as a preliminary research on *in vitro* propagation of *Hornstedtia reticulata* to mass produce genetically uniform plantlets for future conservation plan and ornamental purpose.

Materials and Methods

Materials

Seeds of *Hornstedtia reticulata* were collected at Kampung Segong, Bau, Kuching, Sarawak, Malaysia.

Methods

(a) *Media preparation*

The culture media Gamborg B5 was used in the study (Gamborg *et al.*, 1968). The medium contains 30g/L sucrose and vitamins. After adjusting to pH 5.8±1, medium was solidified using 3g/L gelrite. Culture media were sterilised by autoclaving at 104kPa at 121°C for 20 minutes.

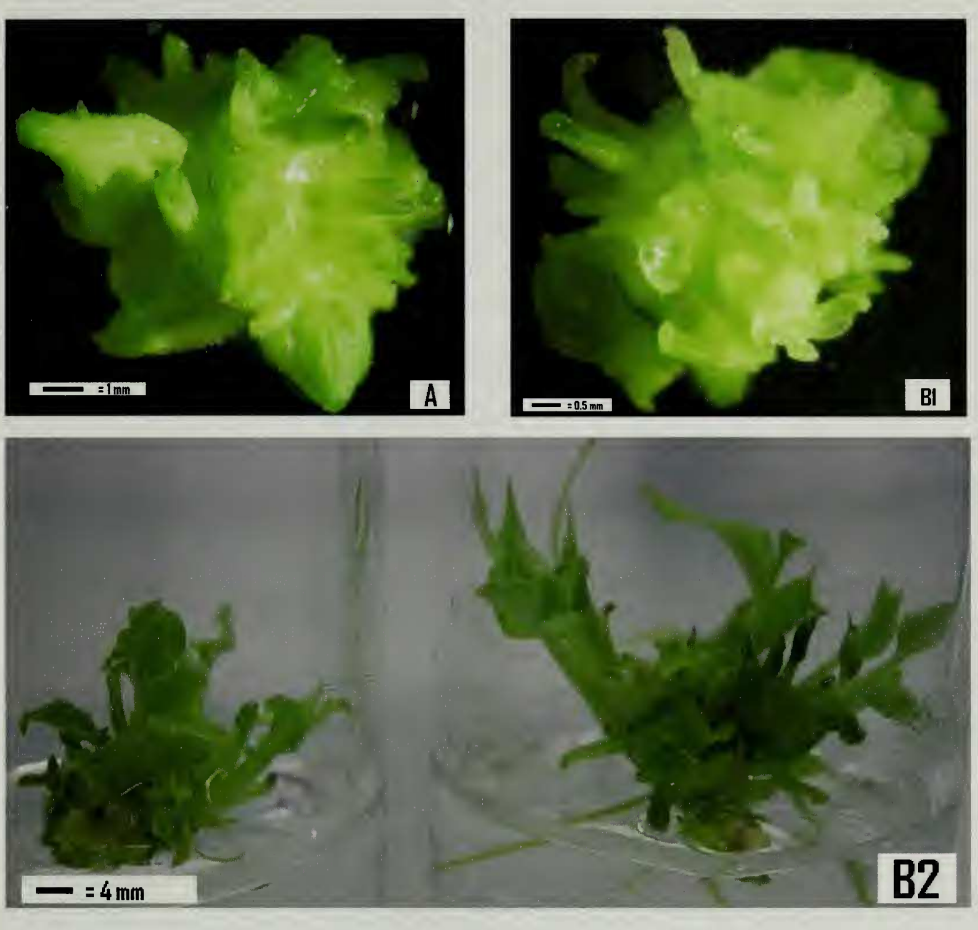
(b) *Surface sterilisation and sowing of seed*

The seeds were soaked in distilled water overnight to remove the mucilage layers. After that, the seeds were soaked in 75% ethanol for one minute before double surface sterilised with 30% Clorox, followed by 15% Clorox, each for 20 minutes. They were then rinsed three times in sterilised distilled water before cultured in B5 media incorporated with 5ml/L PPM and 5mg/L Tetracyclin for seven days. After seven days, the seeds were sown on Gamborg B5 medium

(c) *Induction of multiple shoot formation*

After 12 weeks of culture, 30 seedlings, each 4-5 cm in height, were randomly selected for study on the effects of 6-benzylaminopurine (BAP) alone or with the combination of α-naphthalene acetic acid (NAA) in different concentration. Three replicates were used for each treatment. The roots, the leaves, and the leaf sheaths of seedlings were removed. Then, the meristems of 12 weeks old seedlings, which were cut into approximately 1 cm in length, were used to induce multiple shoots. The explants were monthly subcultured in fresh media with growth regulators incorporated for the first two months and thereafter in the plant growth regulator-free medium. Observation on

the number of explants forming shoots was recorded. Data were subjected to factorial analysis using General Linear Model.



Figures A – B2. Shoot multiplication of *Hornstedtia reticulata*. A. A clump of adventitious shoot buds with primordia excised from meristem explants after 6 weeks of subculturing in media with 3mg/l BAP; B1. A clump of adventitious shoot buds from the basal potion of meristem region in media supplemented with 3mg/l BAP + 0.1mg/l NAA after 8 weeks of subculturing; B2. The clump was cut into two halves and both continued to form cluster of shoots after subcultured onto basal medium.

Results and Discussion

All the treatments having BAP alone, or with the combination of NAA in different concentrations, were able to generate multiple shoots. However, the rate of bud multiplication was significantly different according to the BAP + NAA formulations. Based on Fig. 1, number of shoots produced from explants in 3mg/l BAP was significantly different from the explants

cultured in 2mg/l BAP. Frequency of shoot proliferation was highest at 3mg/L BAP alone. The average number of shoots was 9.67 ± 2.31. Numerous adventitious shoots were observed near the basal potion of the shoot cluster after 12 weeks of subculturing. Multiplication rate in media incorporated with 2mg/L BAP was lowest among the treatments with the mean reading of 4.33 ± 2.31.

In this study, addition of NAA into medium with BAP was proven not efficient in increasing number of multiple shoots. Similar effect was showed in *Zingiber petiolatum*, where maximum shoots regeneration from terminal buds explants was obtained on medium with 4.4 µM BAP alone, while lesser shoots were obtained from the explants that cultured in the media with addition of 0.5 µM NAA (Prathanturarug *et al.*, 2004). Furthermore, similar result was also encountered when the apical meristems of *Curcuma amada* Roxb. and *Zingiber officinale* Rosc. were explanted on media supplemented with BAP alone and BAP incorporated with NAA where the percentage of bud growth were decreased 50% and 67% respectively (Jasrai *et al.*, 2000). Hence, addition of NAA is not recommended for most of the Zingiberaceae plant species.

Observation also illustrated that the explants were started to show significantly increase in number of shoots produced after they were subcultured onto basal medium without PGR. The same finding was found in other Zingiberaceae plant species, such as *Curcuma longa* L. and *Zingiber petiolatum* (Prathanturarug *et al.*, 2003, 2004). Medium for induction of rooting of shoots was not required as the regenerated plantlets produced plentiful roots in the same growth regulators media. Similar observation was seen in plantlets produced from shoot tip explants of *Zingiber officinale* Rosc. in MS media supplemented with BAP + Kinetin treatments (Khatun *et al.*, 2003).

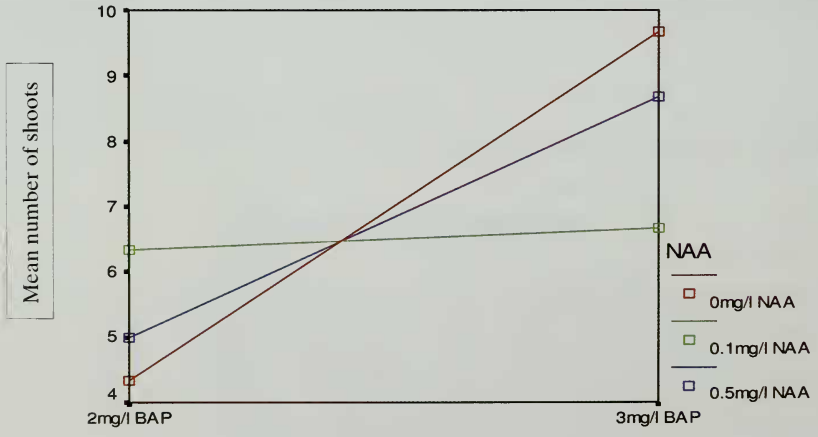


Figure 1. Marginal Means for number of shoots produced.

Conclusion

This report had demonstrated the preliminary result of using tissue culture method as a possible mean for producing large number of true-to-type plantlets of *Hornstedtia reticulata.* BAP alone in concentration of 3mg/l is sufficient enough to micropropagate the plantlets which can be exploited for further research of this species. Currently, the acclimatization has not yet been performed because the regenerated plantlets are kept for further molecular study. Hence, acclimatization will be performed later.

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